云 南 植 物 研 究 2006, 28 (4): 394～398
Acta Botanica Yunnanica

# Effect of 2,4-D on Seedling Physiology and Cytogenetical Studies in *Triticum aestivum* and *Phalaris minor* (Gramineae)

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Abstract: Effects of 2,4-D on seedling growth and chromosomal abnormalities were studied in *Triticum aestivum* and *Phalaris minor*. Seeds were soaked at different concentrations of 2,4-D (0.01%, 0.1%, 1.0%) for 4, 8, 12 and 16 hours. 2,4-D suppressed the germination more severely in *P. minor* than in *T. aestivum*. Shoot and root length was retarded with the increase of concentration and time of treatment in both species. Generally radical was more negatively affected than coleoptile and emergence of radical was not observed at 1.0% concentration at 8, 12, and 16 hours of treatment in *T. aestivum* while in *P. minor* there was a total lack of radical emergence at 1.0% concentration for all durations of treatment. Stiff and curled roots and undifferentiated callus like scutellar tissues were observed in *T. aestivum*, while in *P. minor* the coleoptile obtained was lean, pale green in colour and was lying flat on filter paper. Mitotic index decreased, while chromosomal abnormalities, bridges and laggards were increased with the increase of concentration and soaking time however, laggards were not observed in *T. aestivum*. Clumping and chain formation of chromosomes at metaphase was also noticed in *P. minor*.

Key words: *Triticum aestivum*; *Phalaris minor*; 2,4-D; Seedling grow; Chromosomal abnormality

CLC number: Q945 Document Code: A Article ID: 0253-2700(2006)04-394-05

## Introduction

The chlorinated phenoxy acid compounds are used as herbicides in the form of parent acid or more commonly as salts or esters. The chlorophenoxy herbicides like 2,4-D have profound effects upon the growth and structure of plants. After application of 2,4-D, the growth patterns of plants change (Abeles, 1968; Morrison *et al*., 1981), shoot and root elongation stops (Crafts, 1961; Fedtke, 1982), parenchymatous cells swell, and radial expansion takes place (Pinfield *et al*., 1991). Inhibitory effects of 2,4-D on seed germination were observed in various species (William and Hoagland, 1982; Sathiyamoorthy, 1990). At the cellular level, 2,4-D inhibits cell division (Bingham, 1968), enlargement and differentiation of plant cell and induce mitotic aberrations (Rose, 1970; Mitchison, 1971). Spray of very low doses of 2,4-D result in higher grain yield of wheat (Pandey *et al*., 1998), greater alpha-amylase activity, growth and yield components (Singh *et al*., 1998). 2,4-D is used alone and also in combination with other herbicides to control the weeds including *Phalaris minor* and grain yield was increased significantly in wheat (Sharma and Pandey, 1997; Panwar *et al*., 1998). *Phalaris minor* is considered as an abnoxious weed in wheat fields. The present studies were conducted to see whether 2,4-D only effects the weed or causes any damage to wheat as well, and also to compare the kind and extent of damage to both species at the seedling and cytogenetical levels.

## Materials and Methods

Seeds of *T. aestivum* L. cv Pak 81 and *P. minor* Retz were obtained from National Agriculture Research Centre (NARC) Islamabad. These were surface sterilized with 0.1% mercuric chloride for 10 minutes, and then washed with distilled water. The seeds were subjected to 0.01, 0.1 and 1.0% aqueous solution of 2,4-dichlorophenoxy sodium acetate (2,4-D)

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Received date: 2005-12-26, Accepted date: 2006-03-16

along with control (distilled water) for 4, 8, 12 and 16 hours with constant shaking. The volume of 2,4 -D and distilled water used was three times more than that of seed volume. After treatment seeds were washed in distilled water and then were dried on blotting paper. The treated seeds were grown in petri plates with moistened whatmann filter paper. For seedling physiology, 100 seeds were grown in 4 replications having 25 seeds in each replication. Among the seedling physiology germination, shoot length and root length was noted after 10 days of sowing. Emergence of coleoptile was taken as the criterion for germination. Data was analyzed statistically with the help of software "MSTAT C version 1.3" and means were compared at 0.01-probability level according to Duncan's multiple range test.

For cytogenetical studies 50 seeds for each treatment were sown and root tips about 1.0 cm were excised and fixed in Carnoy's solution (ethanol 3∶1 glacial acetic acid) for 12 hours, washed with distilled water and stored in 70% ethanol. After hydrolysis with 1.0 mol L HCl root tips were stained in 1.0% aceto orcein stain for an hour. Ten root tips were studied for each treatment. To determine the cytotoxic effects of 2,4 -D, mitotic index and chromosomal aberrations were recorded and expressed as the percentage, while the arrested metaphases were determined by deducting the mean of anaphase and telophase from the metaphases. Cells having acentric fragments only were considered as fragments, while chromosomes as bridges either having fragments and or laggards.

## Results and Discussion

Analysis of variance (Table 1) revealed that 2,4 -D concentration, treatment time and genotypes, and their interactions were varied highly significantly ($p < 0.01$). Application of 2,4 -D herbicide inhibited the seed germination percentage, shoot and root length significantly ($p < 0.01$) by increasing the concentration and time of treatment in both species, however in *P. minor* the inhibition in germination was more pronounced (Table 2). These results reflected that different concentrations used were toxic to both species as the germination, root and shoot length was decreased in both the genotypes. Very low dose of 2,4 -D (2 - 4 mg L) is being utilized in tissue culture to obtain callus from seed scutellum in rice (Rashid *et al*. 2001). Emergence of hypocotyl at 1.0% was not observed in *P. minor*, while in wheat beyond 4 hours treatment (Table 2). Phenoxy acids may be inhibitory in one or more of the phases of seedling growth. It has been reported (Crafts, 1961) that 2,4 -D can penetrate the seed coat and is effective as a pre-emergence treatment. The inhibition of seedling growth could be in (i) early germination i.e., the emergence of radical (ii) seedling establishment at the expense of the endosperm or cotyledonary reserve (iii) growth of seedling after the seed reserves are exhausted. These inhibitory effects are brought about by the herbicides on the biochemical and metabolic cycles (Gentner and Burk, 1968). The possible mechanism contributing to herbicidal action of 2, 4 -D was the increased respiration and depletion of food reserves. Auxins like 2, 4 -D are metabolically broken down by the isozyme oxidase with the release of phenols. The accumulation of these phenols in the tissues might inhibit the germination of seeds as indicated by William and Hoagland (1982) and Sathiyamoorthy (1990).

Table 1 Analysis of variance for germination percentage, shoot length and root length in *Triticum aestivum* and *Phalaris minor* after treatment with 2,4 -D

| K source | D.F. | Mean square | | |
|---|---|---|---|---|
| | | Germination | Shoot length | Root length |
| Replications | 3 | 1.448 | 0.194 | 0.089 |
| Genotypes (A) | 1 | 23274.031$^{**}$ | 1663.49$^{**}$ | 81.09$^{**}$ |
| Error (a) | 3 | 2.948 | 0.136 | 0.072 |
| Treatment (B) | 3 | 18703.115$^{**}$ | 321.46$^{**}$ | 94.86$^{**}$ |
| Error (b) | 9 | 1.865 | 0.058 | 0.101 |
| A × B | 3 | 3620.78$^{**}$ | 110.01$^{**}$ | 8.06$^{**}$ |
| Error (c) | 9 | 6.253 | 0.082 | 0.089 |
| Time (C) | 3 | 1794.698$^{**}$ | 43.95$^{**}$ | 4.568$^{**}$ |
| A × C | 3 | 64.365$^{**}$ | 26.85$^{**}$ | 1.865$^{**}$ |
| B × C | 9 | 135.337$^{**}$ | 4.591$^{**}$ | 0.642$^{**}$ |
| A × B × C | 9 | 26.115$^{**}$ | 2.453$^{**}$ | 0.493$^{**}$ |
| Error (d) | 72 | 4.566 | 0.135 | 0.079 |
| Total | 127 | | | |

** : Highly significant at 70.01% level of probability

The marked sensitivity of seedling growth i.e., inhibition of the elongation of radicals or coleoptile is related to herbicide concentration. The data obtained in the present investigation correlates well with the observation of Morrison *et al*. (1981) that the delayed proliferation activity of meristematic cells, the root and shoot growth is retarded. It was further observed that the radical was expanded and swelled while the coleoptiles attained were lean, thin, yellowish green in colour and was lying horizontally on the surface. Abeles (1968) reported that 2,4-D increased ethylene production and suggested that certain morphological changes may be due to enhanced production of ethylene. Mosejev *et al*. (1990) studied the effects of ethephan on cell proliferation and cell expansion in winter rye seedlings. They found that ethylene release from the decomposition of ethephan is a physiologically active process, resulting in growth inhibition. The swelling of hypocotyl in wheat may also be due the endogenous production of ethylene as an impact of the 2,4-D as suggested by Pinfield *et al*. (1991) in swelling of the hypocotyl in *Cucurbeta pepo*.

Table 2 Germination percentage, shoot length and root length effected by 2,4-D at various treatments in *Triticum aestivum* and *Phalaris minor*

| | Germination (%) | | | | |
|---|---|---|---|---|---|
| Hours | 4 | 8 | 12 | 16 | Mean |
| *Triticum aestivum* | | | | | |
| Control | 97.00 A | 99.00 A | 94.00 ABC | 94.00 ABC | 96.00 A |
| 0.01% | 92.00 ABC | 88.00 ABCD | 82.00 BCDEF | 70.50 FGHI | 83.12 B |
| 0.1% | 85.00 ABCDE | 80.00 CDEFG | 74.00 EFGH | 60.00 IJ | 74.75 C |
| 1.0% | 76.50 DEFGH | 66.50 GH | 60.00 IJ | 49.00 JK | 63.00 D |
| Mean | 87.62 A | 83.37 B | 77.50 C | 68.37 D | 79.21 A |
| *Phalaris minor* | | | | | |
| Control | 99.00 A | 97.00 A | 93.00 ABC | 95.00 AB | 96.00 A |
| 0.01% | 77.50 FGH | 65.50 HI | 59.50 IJ | 50.50 J | 63.25 D |
| 0.1% | 37.00 KL | 32.00 LM | 28.50 LMN | 26.00 LMN | 30.87 E |
| 1.0% | 31.00 LM | 19.50 MNO | 16.00 NO | 9.00 O | 18.87 F |
| Mean | 61.12 E | 53.50 F | 49.25 G | 45.12 H | 52.25 B |
| Duration mean | 74.37 A | 68.43 B | 63.37 C | 56.75 D | |
| Treatment mean | 96.00 A | 73.18 B | 52.81 C | 40.93 D | |

| | Shoot length (cm) | | | | |
|---|---|---|---|---|---|
| Hours | 4 | 8 | 12 | 16 | Mean |
| *Triticum aestivum* | | | | | |
| Control | 16.79A | 15.87B | 15.95B | 15.55B | 16.04A |
| 0.01% | 14.41C | 11.52D | 8.81E | 6.40F | 10.29B |
| 0.1% | 10.99D | 8.87E | 6.29F | 5.03G | 7.79C |
| 1.0% | 6.38F | 4.36GH | 3.38IJK | 2.24NO | 4.09D |
| Mean | 12.15A | 10.15B | 8.61C | 7.30D | 9.55A |
| *Phalaris minor* | | | | | |
| Control | 3.85HIJ | 3.97HI | 4.24H | 3.94HI | 4.00D |
| 0.01% | 3.18JKL | 2.99KLM | 2.60LMN | 2.32MNO | 2.77E |
| 0.1% | 2.23NO | 1.67OP | 1.42PQ | 1.23PQR | 1.64F |
| 1.0% | 1.31PQR | 1.12PQR | 0.82QR | 0.63R | 0.97G |
| Mean | 2.64E | 2.43EF | 2.27FG | 2.03G | 2.34B |
| Duration mean | 7.40A | 6.29B | 5.44C | 4.67D | |
| Treatment mean | 10.02A | 6.35B | 4.71C | 2.53D | |

| | Root length (cm) | | | | |
|---|---|---|---|---|---|
| Hours | 4 | 8 | 12 | 16 | Mean |
| *Triticum aestivum* | | | | | |
| Control | 6.25A | 5.30B | 5.96A | 6.12A | 5.91A |
| 0.01% | 4.29C | 3.55D | 3.10DE | 2.52EFG | 3.37B |
| 0.1% | 3.56D | 2.97EF | 2.66EFG | 2.16GHI | 2.84C |
| 1.0% | 2.14GHI | 0.00L | 0.00L | 0.00L | 0.53F |
| Mean | 4.06A | 2.95B | 2.93B | 2.70B | 3.16A |
| *Phalaris minor* | | | | | |
| Control | 2.96EF | 3.08DE | 2.84EF | 2.95EF | 2.96C |
| 0.01% | 2.49FG | 2.26GH | 1.73HIJ | 1.69HIJ | 2.04G |
| 0.1% | 1.60IJ | 1.33JK | 1.18JK | 1.01K | 1.28E |
| 1.0% | 0.00L | 0.00L | 0.00L | 0.00L | 0.00G |
| Mean | 1.76C | 1.67CD | 1.44D | 1.41D | 1.57B |
| Duration mean | 2.91A | 2.31B | 2.18BC | 2.06C | |
| Treatment mean | 4.43A | 2.70B | 2.06C | 0.26D | |

note: Respective means not followed by similar letters are statistically significant at > 0.05% of probability by Duncan's multiple range test

The results reported in this study (Table 2) indicated that hypocotyl was the most sensitive organ to the toxic effects of 2,4-D. A marked effect of 2,4-D on cytogenetics in root tip cells of *T*. *aestivum* and *P*. *minor* was observed. Generally the mitotic index was decreased linearly with the increase in herbicide concentration and soaking time in both the genotypes. However, in *T*. *aestivum* reduction in mitotic index was greater as compared to the mitotic index in *P*. *minor* at various levels of treatment. Chromosomal abnormalities in root tip cells included stickiness at metaphase, bridge formation and lagging of chromosomes at anaphase were due the genotoxic effects of 2,4-D in *P*. *minor* while only first two chromosomal anomalies were reported in *T*. *aestivum* (Fig. 1). These chromosomal abnormalities were tended to increase with the increase of concentration and soaking time (Table 3).

It has been reported that phenoxy acids are stored in the vacuoles of meristematic cells which affect the mitotic activity of cells as an effect on nucleic acid and

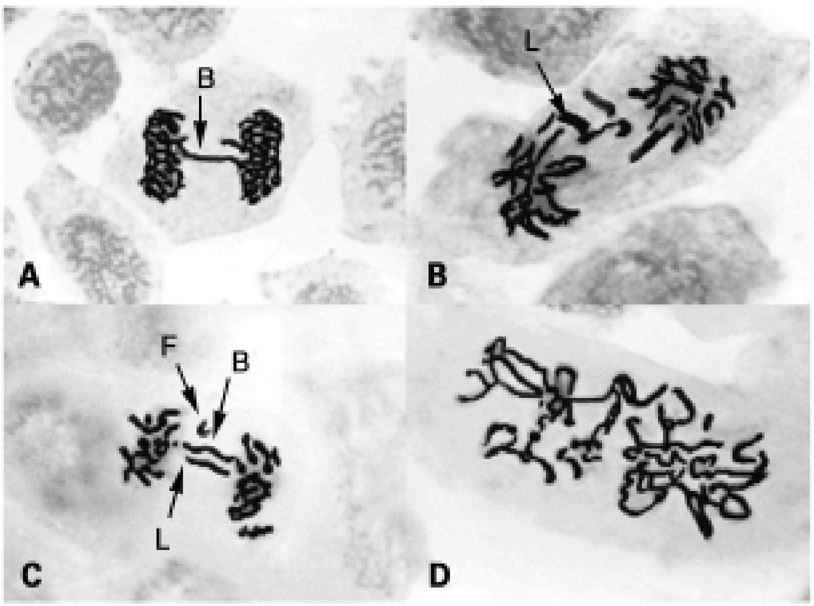

Fig. 1 Various chromosomal abnormalities (shown by arrows) observed in root tip cells after treatment with 2,4-D; A: Bridge formation (B) in *T. aestivum* at anaphase; B: Laggards (L) in *P. minor* at anaphase; C: Bridge formation (B) with laggard (L) and fragment (F) in *P. minor*; D: Stickiness and Clump formation at metaphase in *P. minor*.

proteins (Chen *et al*., 1972). As a consequence of this mechanism the cells of meristem and zone of elongation were stimulated to abnormal swelling by the toxicity of 2, 4-D in wheat. It has been observed (Table 4) that the weedicide 2,4-D arrests the cells at a stage of cell cycle preceding mitosis resulting in a decrease in proportion of cells observed in mitosis relative to those observed in interphase at a particular time as suggested by Mahmound *et al*., (1986). The decrease in prophase frequency implicates first transition point in metaphase before the onset of mitosis. This is based on the length of time from the beginning of treatment. The mitotic index values showed the dose dependent response of the system to the chemical (Table 4).

Table 3 Chromosomal aberrations induced by 2,4-D at various treatments in root tip cells of *Triticum aestivum* and *Phalaris minor*

| | Time (Hours) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Abnormal cells (%) | | | | Bridges (%) | | | | Laggards (%) | | | |
| | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 |
| *Triticum aestivum* | | | | | | | | | | | | |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.01% | 0.17 | 0.35 | 0.41 | 0.58 | 0.17 | 0.35 | 0.41 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1% | 0.30 | 0.40 | 0.47 | 0.59 | 0.30 | 0.40 | 0.47 | 0.59 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.0% | 0.39 | —— | —— | —— | 0.39 | —— | —— | —— | —— | —— | —— | —— |
| *Phalaris minor* | | | | | | | | | | | | |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.01% | 1.66 | 1.87 | 1.87 | 2.14 | 1.05 | 1.24 | 1.23 | 1.48 | 0.60 | 0.62 | 0.63 | 0.65 |
| 0.1% | 2.04 | 2.33 | 2.34 | 2.80 | 1.35 | 1.60 | 1.62 | 1.96 | 0.69 | 0.72 | 0.72 | 0.84 |
| 1.0% | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— |

note: —— Roots not developed

Table 4 Effect of 2,4-D on mitotic index and arrested metaphases in root tip cells of *Triticum aestivum* and in *Phalaris minor*

| | Mitotic index (%) | | | | Arrested metaphases (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 4 | 8 | 12 | 16 |
| *Triticum aestivum* | | | | | | | | |
| Control | 18.13(100.00) | 18.40(100.00) | 18.91(100.00) | 18.21(100.00) | 2.22(100.00) | 2.15(100.00) | 2.00(100.00) | 2.25(100.00) |
| 0.01% | 91.94 | 84.13 | 75.03 | 71.44 | 193.24 | 174.40 | 215.50 | 231.55 |
| 0.1% | 88.25 | 82.44 | 74.61 | 73.70 | 131.53 | 200.46 | 292.00 | 264.88 |
| 1.0% | 82.90 | —— | —— | —— | 250.90 | —— | —— | —— |
| *Phalaris minor* | | | | | | | | |
| Control | 24.76(100.00) | 24.83(100.00) | 25.09(100.00) | 24.32(100.00) | 0.70(100.00) | 0.78(100.00) | 0.74(100.00) | 0.80(100.00) |
| 0.01% | 94.62 | 93.40 | 87.96 | 83.26 | 230.00 | 265.38 | 195.94 | 167.50 |
| 0.1% | 86.82 | 95.56 | 79.35 | 77.05 | 78.57 | 101.28 | 81.08 | 75.00 |
| 1.0% | —— | —— | —— | —— | —— | —— | —— | —— |

note: —— Roots not developed

The frequency of metaphase tended to be increased with the increase in concentration and soaking time in *T. aestivum*, while in *P. minor* increase in metaphase frequency was found at lowest dose of 2,4-D and at higher concentration the frequency of metaphase was decreased. From the results (Table 4) it becomes apparent that 2,4-D altered the movements of cells through the nuclear cycle. The rise in metaphase frequency in *T*. *aestivum* suggests the presence of transition point at the end of metaphase. This might be due to the fact that 2,4-D can inhibit the assembly of spindle apparatus (Unrau, 1954; Rose, 1970). However, in the case of *P*. *minor* stickiness of chromosomes at lowest dose was found due to which the increase in metaphase was recorded. This stickiness of chromosomes might have arisen as an effect on the proteins involved in chromosome condensation as reported by Butts and Fang (1956). It has been investigated (Mitchison, 1971) that specific proteins are involved for the condensation of chromosomes and their movement through mitosis. Many of these proteins play a structural role, while others are concerned with energy requirement influencing the normal progression of nuclear division. As a result, the frequency of variants associated with chromosome condensation, movement and organization tended to increase in the treated root tip cells (Baszczynski *et al*., 1980). From these studies it has been concluded that *P*. *minor* was more severely affected by 2,4-D as compared to *T*. *aestivum* as far as the seedling physiology and the chromosomal variants are concerned.

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